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(71) Applicant *(for all designated States except US)*: BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: BURKLY, Linda, C. [US/US]; 34 Winthrop Street, West Newton, MA 02165 (US).

(74) Agent: MIAO, Emily; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).

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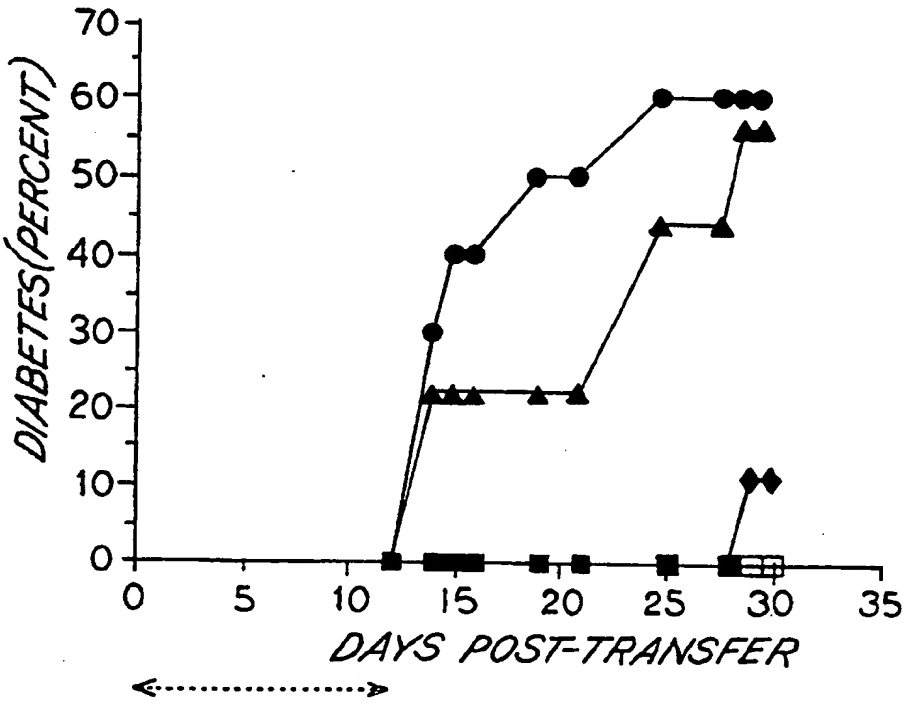
(57) Abstract

A method for the prevention of insulin dependent (type I) diabetes. The method comprises administration of an antibody, polypeptide or other molecule recognizing VLA4.



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TREATMENT FOR INSULIN DEPENDENT DIABETES

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/029,330, filed February 9, 1993.

FIELD OF THE INVENTION

The present invention relates to a treatment for insulin dependent (type-I) diabetes. More particularly, this invention relates to the use of antibodies recognizing the integrin VLA4 (very late antigen 4) in the prevention of diabetes.

BACKGROUND OF THE INVENTION

Insulin dependent diabetes (also termed type-I diabetes and formerly juvenile onset diabetes mellitus) has been classified during the past two decades as a chronic autoimmune disease. In this disorder, cells producing insulin (β cells) within the pancreatic islets are selectively targeted and destroyed by a cellular infiltrate of the pancreas. This inflammatory infiltrate affecting the islets has been termed insulitis. Cells producing insulin comprise the majority of islet cells but less than 2% of the total pancreatic mass (Castano and Eisenbarth, 1990, [1]; Fujita et al., 1982 [2]; Foulis et al., 1986 [3]). The development of type I diabetes can conceptually be divided into six stages, beginning with genetic susceptibility and ending with complete β cell destruction (Eisenbarth, 1986 [4]). Stage I is genetic susceptibility, which is a necessary but insufficient condition for development of the disease. A hypothetical triggering event (Stage II) leads to active autoimmunity against β cells (Stage III). In Stage III, the β cell mass is hypothesized to decline

and immunologic abn rmalities such as autoantibodies directed against insulin and islet cytcplasmic antigens are f und. Stimulated insulin s cretion is still preserved at this stage. Over a period of years, however, the progressive loss of β cells leads to diminished insulin secretion with intravenous glucose tolerance tests (IVGTT) while the individual is still normoglycemic (Stage IV). Overt diabetes (i.e., diabetes onset or clinical manifestation of disease characterized by hyperglycemia) is Stage V, and can develop years later when approximately 90% of pancreatic β cells are destroyed. In Stage V when overt diabetes is first recognized, some residual insulin production remains (as demonstrated by the presence of the connecting peptide of proinsulin, C peptide, in the serum) but the individual usually requires exogenous insulin for life. Finally, in Stage VI, even the remaining β cells are destroyed and C peptide can no longer be detected in the circulation.

While the initiating factor(s) and specific sequence of events leading to diabetes, including the relative importance of different cell types and cytokines, are still widely debated, a key role is generally recognized for self-antigen reactive T cells (Miller et al., 1988 [5]; Harada and Makino, 1986 [6]; Koike et al., 1987 [7]; Makino et al., 1986 [8]). In addition to T lymphocytes, insulitis is characterized by macrophages, dendritic cells (Voorbij et al., 1989 [9]) and B cells, which may serve as professional antigen presenting cells (APC). Macrophages may also destroy islet β cells themselves by release of cytokines or free radicals (Nomikos et al., 1986 [10]). Thus, autoimmune diabetes relies upon both cellular migration and immune stimulation of newly resident cells.

Cell trafficking to inflammatory sites is regulated by accessory mol cules LFA-1, MAC-1 and VLA4 (Larson and Springer, 1990 [11]; Hemler et al., 1990 [12]) on the surface of lymphocytes (LFA-1, VLA4) and macrophages (Mac-1, VLA4), and by their counter-ligands ICAM (for LFA-1 and MAC-1), and VCAM (for VLA4) which are unregulated by cytokines on vascular endothelium (Larson and Springer, 1990 [11]; Lobb, 1992 [13]; Osborn, 1990,[14]). In addition, VLA4 binds to an extracellular matrix component, the CS-1 domain of fibronectin (FN) (Wayner et al., 1989 [15]). The relative importance of these pathways, for example, LFA-1 and VLA4 on lymphocytes or MAC-1 and VLA4 on monocytes, in controlling cell migration is still a subject of investigation. *In vitro* data suggest that the differential use of these pathways appears to depend upon the activation status of both the leukocytes and endothelial cells (Shimizu et al., 1991 [16]). Their ability to control cell migration to inflammatory sites *in vivo* has been directly demonstrated with monoclonal antibodies (mAbs) to ICAM, MAC-1 or VLA4 inhibiting various animal models of disease (Barton et al., 1989 [17], phorbol ester-induced rabbit lung inflammation; Issekutz and Issekutz, 1991 [18], delayed type hypersensitivity; Issekutz, 1991 [19], adjuvant-induced arthritis; Yednock et al., 1992 [20], transfer of experimental allergic encephalomyelitis (EAE); Lobb, 1992 [21], asthma).

ICAM and VCAM are also found on the surface of macrophages and dendritic cells in lymphoid tissues (Dustin et al., 1986 [22]; Rice et al., 1990 [23]; Rice et al., 1991 [24]). Their distribution on these professional APC is consistent with functional data indicating a role for LFA-1 and VLA4 in T cell

activation (Shimuzu et al., 1990 [25], Burkly et al., 1991 [26]). Howev r, num rous oth r receptor-ligand pairs including CD4/ MHC class II and CD8/MHC class I (Rudd et al., 1989 [27]), CD2/LFA-3 (Moingeon et al., 1989 [287]), CD28/B7 (Harding et al., 1992 [29]) may also support adhesion or costimulate T cells during T/APC or T/target cell interactions. The specific contributions of these numerous pathways in the development of diabetes is unresolved. Because there are multiple molecular pathways for cell adhesion and T cell activation, it is not possible to predict whether intervention in one or more of these pathways might affect onset or severity of diabetes disease, and, in particular, which of these pathways are crucial or relevant to the disease process.

Antibodies directed to T cells have been utilized in murine and rat models for spontaneous diabetes and adoptive transfer of diabetes to deplete T cells and thus prevent disease (see, e.g., Harada and Makino, 1986 [6], anti-Thy 1.2; Koike et al., 1987 [7], Miller et al., 1988 [5] and Shizuru et al., 1988 [30], anti-CD4; Barlow and Like, 1992 [31], anti-CD2; Like et al., 1986 [32], anti-CD5 and anti-CD8). In addition, an antibody directed to the complement receptor type 3 (CR3) molecule or MAC-1 on macrophages has been utilized to prevent macrophage and T cell infiltration of pancreatic tissue in a murine adoptive transfer model of disease (Hutchings et al., 1990 [33]). It is unknown whether VLA4 is relevant to insulitis or to the activity of islet-specific cells after localization in the pancreas.

Current treatment protocols suggested for type I diabetes have included certain immunomodulatory drugs summarized by Federlin and Becker [34] and references cited therein. A long prediabetic period with

immun logic abnormalities and progressive β cell destructi n suggests it may b p ssibl to halt β c ll l ss with immune intervention (Castano and Eisenbarth, 1990 [1]).

Suggested agents/protocols have included certain immunomodulatory and immunosuppressive agents: levamisol, theophyllin, thymic hormones, ciamexone, anti-thymocyte globulin, interferon, nicotinamide, gamma globulin infusion, plasmapheresis or white cell transfusion. Agents such as cyclosporin A and azathioprine which impair T cell activation and T cell development, respectively, have been used in clinical trials (Zielasek et al., 1989 [35]). The most promising results have been achieved with cyclosporin A (Castano and Eisenbarth, 1990 [1]). Federlin and Becker, 1990 [34] suggest, however, that cyclosporin A may not be recommended for general or long-term use because of toxic side effects, at least when given in higher doses. Higher doses of cyclosporin, or in combination with other immunosuppressive drugs, or both, have been associated with the development of lymphoma and irreversible kidney damage (Eisenbarth, 1986 [4]; Eisenbarth, 1987 [36]) Additional studies on other suggested agents are necessary to assess safety and efficacy. Even the cyclosporin A studies show that its efficacy in maintaining remission of diabetes is for one year in about 30-60% of new onset diabetes. Within 3 years, however, remissions are almost invariably lost (Castano and Eisenbarth, 1990 [1]). Treatment protocols after onset of disease are particularly problematic, since, for example, at the time diabetes is diagnosed in humans, insulitis has typically progressed already to a loss of more than 80% of the β cells. Thus, it is possible that cyclosporin A may be preventing further β cell destruction, but so

few β cells may be present at the onset of the diabetes that they cannot maintain a non-diabetic state over time (Castano and Eisenbarth, 1990 [1]). Suppression of insulitis and/or prevention of disease may be more successful if the treatment could start at an earlier phase, i.e., before disease onset.

There are two major prerequisites in order to develop any preventative treatment for diabetes disease: (1) the ability to accurately identify the prediabetic individual and (2) the development of safe, specific and effective preventive treatments. Significant progress has been made in identifying prediabetic individuals, however, much work remains in the development of safe, specific and effective preventive treatments as discussed and reviewed by Eisenbarth and colleagues (see, e.g., Ziegler and Eisenbarth, 1990 [37]; Ziegler et al., 1990 [38]; Ziegler et al., 1990 [39]). It has been possible to identify certain risk factors and at-risk groups for type I diabetes and thus to predict individuals most likely to go on to clinical disease and to estimate the approximate rate of disease onset in these individuals. The ability to identify individuals with susceptibility to diabetes or to predict type I diabetes in the pre-clinical stage by the combination of genetic (HLA typing), immunological (islet and insulin autoantibodies) and metabolic (first phase insulin secretion to intravenous glucose preceding the development of hyperglycemia) markers makes the identification and use of prophylactic immunotherapeutic drugs and protocols possible during the evolution of the autoimmune disease process when β cell destruction is only partial. To date, there has been little success, however, in treating human diabetes. Generally, because human treatment has been

used only after onset of the disease, treatment was followed by a temporary complet or partial remission only in a certain number of patients. Since immunosuppressive mechanisms may prevent insulitis and/or diabetes, there is a need for immunosuppressive components for use in the prediabetic stage. In particular, there is a need for safer and more specifically acting compounds, e.g., monoclonal antibodies, which inhibit entry of effector cells into the pancreas or function of those cell which may have already entered the islets of Langerhans.

It has now been surprisingly discovered that administering an anti-VLA4 antibody significantly reduced the incidence of diabetes, in a rodent model of diabetes disease. The NOD mouse model of diabetes is a well established model directly comparable to human type-I diabetes. Using an adoptively transferred disease experimental protocol, irradiated non-diabetic NOD mice were administered splenocytes from spontaneously diabetic NOD mice for the acute transfer of the disease. These splenocytes were treated with anti-VLA4 antibody before administration and the recipients were also treated for various periods of time after the transfer with anti-VLA4 antibody.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides novel methods for the treatment of insulin dependent (type-I) diabetes in a prediabetic. In particular, the present invention provides a method for the prevention of insulin dependent diabetes comprising the step of administering to a prediabetic individual an anti-VLA4 antibody, such as antibody HP1/2 or a humanized anti-VLA4 antibody derived from HP1/2. Also contemplated is the use of analogous antibodies, antibody fragments, soluble proteins and small molecules that mimic the action of anti-VLA4 antibodies in the treatment of diabetes. In addition, the present invention provides a method for the treatment of diabetes by administering to a mammal, including a human, with a susceptibility to diabetes an antibody capable of binding to the α4 subunit of VLA4 in an amount effective to provide inhibition of the onset of diabetes. Also contemplated is the use of recombinant and chimeric antibodies, fragments of such antibodies, polypeptides or small molecules capable of binding α4/VLA4. Also contemplated are soluble forms of the natural binding proteins for VLA 4, including soluble VCAM-1, VCAM-1 peptides or VCAM-1 fusion proteins as well as fibronectin, fibronectin having an alternatively spliced non-type III connecting segment and fibronectin peptides containing the amino acid sequence EILDV or a similar conservatively substituted amino acid sequence. These agents will act by competing with the cell-surface binding protein for VLA4.

BRIEF DESCRIPTION OF THE DRAWINGS

Figur 1 is a graph depicting th effect of anti-VLA4 antib dy (R1-2) and contr ls on prevention of diabetes after adoptive transfer of spleen cells; the frequency of recipients which became diabetic and day of disease onset are shown for transfer of $2x10^7$ splenocytes from diabetic (D) NOD donors without treatment (closed circles), with a non-specific rat IgG2b treatment (closed triangles), and with R1-2 anti-VLA4 treatment (closed diamonds), as well as for transfer of splenocytes from nondiabetic (Y) NOD donors (open squares); the splenocytes were transferred with R1-2 or rat IgG2b or without mAb, and then R1-2 or rat IgG2b was injected every other day through day 12 post transfer (n=8-10 for all groups).

Figure 2 is a graph depicting the effect of anti-VLA4 antibody (R1-2) and controls on prevention of diabetes after adoptive transfer of spleen cells; the frequency of recipients which became diabetic and day of disease onset are shown for transfer of $3x10^7$ splenocytes from diabetic (D) NOD donors without treatment (closed circles), with a non-specific rat IgG2b treatment (closed triangles), and with R1-2 anti-VLA4 treatment (closed diamonds), as well as for transfer of splenocytes from nondiabetic (Y) NOD donors (open squares); the splenocytes were transferred with R1-2 or rat IgG2b or without mAb, and then R1-2 or rat IgG2b was injected every 3.5 days through day 25 post transfer (n=4-5 for all groups).

Figure 3 is a graph depicting the effect of anti-VLA4 antibody (R1-2) and controls on prevention of diabetes after adoptive transfer of spleen cells; the frequency of recipients which became diabetic and day of disease onset are shown for transfer of $2-3x10^7$ splenocytes from diabetic (D) NOD donors without

treatment (closed circles), with a non-specific rat IgG2b tr atm nt (closed triangles), and with R1-2 anti-VLA4 tr atment (closed diamonds), as w ll as for transfer of splenocytes from nondiabetic (Y) NOD donors (open squares) or for PBS alone (open circles); the splenocytes were transferred with R1-2 or rat IgG2b or without mAb, and then R1-2 or rat IgG2b was injected every 3.5 days through day 25 post transfer (n=5 for all groups).

Figure 4 is a bar graph depicting the effect of anti-VLA4 antibody (R1-2) and controls on the degree of insulitis after adoptive transfer of spleen cells; the frequency of uninfiltrated islets (Grade 0-I infiltrate, stipled bar) and infiltrated islets (Grade II-IV insulitis, solid bar) were quantitated and shown after transfer of cells treated with R1-2, rat IgG2b r without mAb, and then R1-2 or rat IgG2b injected every 3.5 days through day 25 with mice sacrificed when diabetic or on day 26 post-transfer. Pancreatic sections from n=4-5 mice were scored for each experimental group, i.e., Y→Y (non-diabetic donor cells) or D→Y (diabetic donor cells) into non-diabetic (Y) recipients with no mAb treatment, treatment with rat IgG2b or treatment with R1-2.

Figure 5 is a bar graph depicting the effect of anti-VLA4 antibody (R1-2) and controls on the degree of insulitis after adoptive transfer of spleen cells; the frequency of uninfiltrated islets (Grade 0-I infiltrate, stipled bar) and infiltrated islets (Grade II-IV insulitis, solid bar) were quantitated and shown after transfer of cells treated with R1-2, rat IgG2b or without mAb, and then R1-2 or rat IgG2b injected every other day through day 12 post-transfer, then maintained without further mAb injection until sacrificed when diabetic or on day 29 post-transfer. Pancreatic

sections from n=4-5 mice were scored for each experimental group, i.e., Y→Y (non-diabetic donor cells) or D→Y (diabetic donor cells) into non-diabetic (Y) recipients with no mAb treatment, treatment with rat IgG2b or treatment with R1-2.

Figure 6 is a graph depicting the effect of anti-VLA4 antibody (R1-2) and controls on prevention of diabetes in a spontaneous disease model for diabetes; the frequency of recipients which became diabetic and day of disease onset are shown for NOD mice without treatment (closed squares), with a non-specific rat IgG2b treatment (closed circles), and with R1-2 anti-VLA4 treatment (closed triangles); R1-2 or rat IgG2b was injected for 8 weeks in NOD mice twice weekly from week four to week twelve of age.

Figure 7 is a graph depicting the effect of VCAM 2D-IgG fusion protein and controls on prevention of diabetes after adoptive transfer of spleen cells; the frequency of recipients which became diabetic and day of disease onset are shown for transfer of $2x10^7$ splenocytes from diabetic (D) NOD donors with an irrelevant rat LFA-3Ig fusion protein treatment (closed squares), and with VCAM 2D-IgG treatment (open circles) or of recipients which recieved PBS alone without cells transferred (closed triangles); the splenocytes were transferred with VCAM 2D-IgG or rat LFA-3Ig, and then VCAM 2D-IgG or rat LFA-3Ig was injected every other day through day 17 post-transfer (n = 5 for all groups).

Figure 8 is a schematic depicting structure of VCAM 2DIgG fusion protein described in Example 5. VCAM 2D-IgG is a soluble form of the ligand for VLA4 (VCAM1) and consists of the two N-terminal domains of VCAM1 fused to the human IgG1 heavy chain constant region sequences (Hinges, C_H2 and C_H3).

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to a treatment including the prevention of insulin dependent (type I) diabetes. More particularly, the invention relates to the use of antibodies to VLA4 in the treatment of diabetes in a prediabetic individual. The term "prediabetic" is intended to mean an individual at risk for the development of diabetes disease (e.g., genetically predisposed) at any stage in the disease process prior to overt diabetes or diabetes onset. The term "diabetic" is intended to mean an individual with overt hyperglycemia (i.e., fasting blood glucose levels ≥ 250 mg/dL). The term "overt diabetes" or "diabetes onset" is intended to mean a disease state in which the pancreatic islet cells are destroyed and which is manifested clinically by overt hyperglycemia (i.e., fasting blood glucose levels ≥ 250 mg/dL).

In the first aspect, the invention provides a method of treatment of diabetes comprising the step of administering a composition capable of binding to, including blocking or coating, the VLA4 antigens on the surface of VLA4-positive cells, including lymphocytes and macrophages. For purposes of the invention, the term "binding to VLA4 antigens" is intended to mean reacting with VLA4 antigens on cells and thereby interfering with interactions between VLA4 antigens and either VCAM-1 or fibronectin on the surface of other cells or thereby inducing a change in the function of the VLA4-positive cells. As demonstrated herein, such binding, including blocking or coating, of VLA4 antigens results in a prevention in or protection against the incidence of diabetes. This demonstration utilized a monoclonal antibody against VLA4 as a binding agent which effectively blocked or coated the VLA4 antigens. Those skilled in the art will recognize

that, given this demonstration, any agent that can bind to, including those that can block or coat, VLA4 antigens can be successfully used in the method of the invention. Thus, for purposes of the invention, any agent capable of binding to VLA4 antigens on the surface of VLA4-bearing cells and which may effectively block or coat VLA4 antigens, is considered to be an equivalent of the monoclonal antibody used in the examples herein. For example, the invention contemplates as binding equivalents at least peptides, peptide mimetics, carbohydrates and small molecules capable of binding VLA4 antigens on the surface of VLA4-bearing cells.

In a preferred embodiment, the agent that is used in the method of the invention to bind to, including block or coat, cell-surface VLA4 antigens is a monoclonal antibody or antibody derivative. Preferred antibody derivatives for treatment, in particular for human treatment, include humanized recombinant antibodies, chimeric recombinant antibodies, Fab, Fab', $F(ab')_2$ and F(v) antibody fragments, and monomers or dimers of antibody heavy or light chains or intermixtures thereof. Thus, monoclonal antibodies against VLA4 are a preferred binding agent in the method according to the invention.

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with whole cells expressing a given antigen, e.g., VLA4, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen. (See, generally, Kohler et al., 1975 [40]).

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen

depend on the sp cies of mammal immunized, its immune status, the body weight f th mammal, etc. Typically, the immunized mammals ar bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, anti-VLA4 antibodies may be identified by immunoprecipitation of ^{125}I-labeled cell lysates from VLA4-expressing cells. (See, Sanchez-Madrid et al. 1986 [41] and Hemler et al. 1987 [42]). Anti-VLA4 antibodies may also be identified by flow cytometry, e.g., by measuring fluorescent staining of Ramos cells incubated with an antibody believed to recognize VLA4 (see, Elices et al., (1990) [43]). The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive for the presence of anti-VLA4 antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants. For example, hybridomas prepared to produce anti-VLA4 antibodies may be screened by testing the hybridoma culture supernatant for secreted antibodies having the ability

t bind t a rec mbinant α_4-subunit-expressing cell lin , such as transf cted K-562 cells (se , Elic s t al. [43]).

To produce anti-VLA4 antibodies, hybridoma cells that tested positive in such screening assays were cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cells are well known. The conditioned hybridoma culture supernatant may be collected and the anti-VLA4 antibodies optionally further purified by well-known methods.

Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of an unimmunized mouse. The hybridoma cells proliferate in the peritoneal cavity, secreting the antibody which accumulates as ascites fluid. The antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

Several mouse anti-VLA4 monoclonal antibodies have been previously described (see, e.g., Sanchez-Madrid et al., 1986 [41]; Hemler et al., 1987 [42]; Pulido et al., 1991 [44]). These anti-VLA4 monoclonal antibodies such as HP1/2 and other anti-VLA4 antibodies (e.g., mAb HP2/1, HP2/4, L25, P4C2, P4G9) capable of recognizing the α chain of VLA4 will be useful in the methods of treatment according to the present invention. Anti-VLA4 antibodies that will recognize the VLA-α_4 chain epitopes involved in binding to VCAM-1 and fibronectin ligands (i.e., antibodies which can bind to VLA4 at a site involved in ligand recognition and block VCAM-1 and fibronectin binding) are preferred. Such antibodies have been defined as B epitope-specific

antibodies (B1 or B2) (see, Pulido et al. (1991) [36]) and are preferred anti-VLA4 antibodies according to the present invention. The R1-2 antibody used as described herein is a B epitope type antibody.

Human monoclonal antibodies against VLA4 are another preferred binding agent which may block or coat VLA4 antigens in the method of the invention. These may be prepared using *in vitro*-primed human splenocytes, as described by Boerner et al., 1991 [45]. Alternatively, they may be prepared by repertoire cloning as described by Persson et al., 1991 [46] or by Huang and Stollar, 1991 [47]. Another preferred binding agent which may block or coat VLA4 antigens in the method of the invention is a chimeric recombinant antibody having anti-VLA4 specificity and a human antibody constant region. Yet another preferred binding agent which may block or coat VLA4 antigens in the method of the invention is a humanized recombinant antibody having anti-VLA4 specificity. Humanized antibodies may be prepared, as exemplified in Jones et al., 1986 [48]; Riechmann, 1988, [49]; Queen et al., 1989 [50]; and Orlandi et al., 1989 [51]. Preferred binding agents including chimeric recombinant and humanized recombinant antibodies with B epitope specificity have been prepared and are described in co-pending and co-assigned U.S. Patent Application Serial No. 08/004,798, filed January 12, 1993 [52]. The starting material for the preparation of chimeric (mouse V - human C) and humanized anti-VLA4 antibodies may be a murine monoclonal anti-VLA4 antibody as previously described, a monoclonal anti-VLA4 antibody commercially available (e.g., HP2/1, Amac International, Inc., Westbrook, Maine), or a monoclonal anti-VLA4 antibody prepared in accordance with the teaching herein. For example, the variable regions of

the h avy and light chains f th anti-VLA4 antibody HP1/2 have been cloned, sequenc d and expr ssed in combination with c nstant regions of human immunoglobulin heavy and light chains. Such a chimeric HP1/2 antibody is similar in specificity and potency to the murine HP1/2 antibody, and may be useful in methods of treatment according to the present invention. The HP1/2 V_H DNA sequence and its translated amino acid sequences are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The HP1/2 V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 3 and SEQ ID NO: 4, respectively. Similarly, humanized recombinant anti-VLA4 antibodies may be useful in these methods. A preferred humanized recombinant anti-VLA4 antibody is an AS/SVMDY antibody, for example, the AS/SVMDY antibody produced by the cell line deposited with the ATCC on November 3, 1992 and given accession no. CRL 11175. The AS/SVMDY humanized antibody is at least equipotent with or perhaps more potent than the murine HP1/2 antibody. The AS V_H DNA sequence and its translated amino acid sequences are set forth in SEQ ID NO: 5 and SEQ ID NO: 6, respectively. The SVMDY V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 7 and SEQ ID NO: 8, respectively.

Those skilled in the art will recognize that any of the above-identified antibody or antibody derivative binding agents can also act in the method of the invention by binding to the receptor for VLA4, and may block or coat the cell-surface VLA4 antigen. Thus, antibody and antibody derivative binding agents according to the invention may include embodiments having binding specificity for VCAM-1 or fibronectin, since these molecules appear to either be important in the adhesion cells or the extracellular matrix or

interfere with traffic of cells through tissues and blood.

Alternatively, the binding agents used in the method according to the invention may not be antibodies or antibody derivatives, but rather may be soluble forms of the natural binding proteins for VLA4. These binding agents include soluble VCAM-1, VCAM-1 peptides, or VCAM-1 fusion proteins as well as fibronectin, fibronectin having an alternatively spliced non-type III connecting segment and fibronectin peptides containing the amino acid sequence EILDV or a similar conservatively substituted amino acid sequence. These binding agents will act by competing with the cell-surface binding protein for VLA4.

In this method according to the first aspect of the invention, VLA4 binding agents are preferably administered parenterally. The VLA4 binding agents are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier, which may be any of the numerous well known carriers, such as water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, or combinations thereof. Preferably, the VLA4 binding agent, if an antibody or antibody derivative, will be administered at a dose ranging between about 0.1 mg/kg body weight/day and about 20 mg/kg body weight/day, preferably ranging between about 0.1 mg/kg body weight/day and about 10 mg/kg body weight/day and at intervals of every 1-14 days. For non-antibody or antibody derivative binding agents, the dose range should preferably be between molar equivalent amounts to these amounts of antibody. Preferably, an antibody composition is administered in an amount effective to provide a plasma level of antibody of at least 1 μg/ml. Optimization of dosages can be determined by

administration of the binding ag nts, followed by ass ssment f th coating f VLA4-p sitive cells by the agent over time after adminis r d at a given dose *in vivo*. Peripheral blood mononuclear cells contained in a sample of the individual's peripheral blood should be probed for the presence of the agent *in vitro* (or *ex vivo*) using a second reagent to detect the administered agent. For example, this may be a fluorochrome labelled antibody specific for the administered agent which is then measured by standard FACS (fluorescence activated cell sorter) analysis. Alternatively, presence of the administered agent may be detected *in vitro* (or *ex vivo*) by the inability or decreased ability of the individual's cells to bind the same agent which has been itself labelled (e.g., by a fluorochrome). The preferred dosage should produce detectable coating of the vast majority of VLA4-positive cells. Preferably, coating is sustained in the case of a monoclonal antibody or monoclonal antibody derivative for a 1-14 day period.

In practicing this invention, treatment with VLA4 binding agents is preferrably continued for as long as the prediabetic subject maintains a stable normoglycemic plasma level and a stable prediabetic state as reflected by a number of known markers as described above. In the Examples which follow, it has been found that anti-VLA4 mAb, e.g., R1-2 mAb, administration prevented diabetes onset during treatment and that the residual beneficial results of treatment were extended as long as two months following cessation of R1-2 treatment. To sustain the full protective effect of the VLA4 binding agent against diabetes onset, however, continuous treatment with the binding agents is preferred.

The method of the present invention comprises administering to a pre diabetic individual a composition comprising an anti-VLA4 antibody. The examples below set forth the results observed in a rodent model of disease. These results demonstrate a protective effect of anti-VLA4 antibody in disease onset in the acute transfer model of the disease. The non-obese diabetic (NOD) mouse has become an important model of type I or insulin dependent diabetes mellitus since its introduction by Makino et al., 1980 [7] and has been documented as a particularly relevant model for human diabetes (see, e.g., Castano and Eisenbarth [1], Miller et al., 1988 [5], Hutchings et al., 1990 [33] and references cited therein). That the diabetic syndromes displayed in the NOD mouse and human are similar has been shown by several lines of evidence. For example, in both the NOD mouse and human [1], there is a strong genetic association of diabetes with loci of the major histocompatibility complex. In addition, for example, in both species, an autoimmune pathogenesis is evidenced by (i) the presence of lymphocytic inflammation in the pancreatic islets (i.e., insulitis) that appears to mediate the selective destruction of β cells, (ii) the presence of anti-islet cell antibodies, and (iii) the modulating effects of cyclosporin A. Further evidence in the NOD mouse for an autoimmune etiology of diabetes disease is (i) the ability to transfer diabetes with spleen cells (including purified splenic T cells) from diabetic donors, (ii) prevention of diabetes by *in vivo* treatment with antibodies specific for T cells, and (iii) failure of a thymic nude mice with NOD genetic background to develop moulitis or diabetes (see, e.g., Miller et al., 1988 [5], Hutchings et al., 1990 [33] and references cited therein).

Although th precise vents resulting in diabetes remain uncl ar, in the NOD m use a pr gressive inflammatory r sponse in the pancreas app ars to be the initial histological lesion which begins as a periductal /perivascular mononuclear cell infiltrate at 3-4 weeks of age. At about 4-6 weeks of age, insulitis may be observed and beginning at about 12 weeks of age, overt diabetes (i.e., consistent values of 1+ or higher using a Testape (Eli Lilly, Indianapolis, IN) assay for glycosuria or greater than 250 mg/dL if plasma glucose is monitored) occurs. To avoid variations in the immune status of the animals, the NOD mice are obtained from a specific pathogen-free colony and exhibit stable, high incidence of diabetes of about 80% of females and 20% of males which typically become diabetic by about 20 weeks of age. The preferred source for the NOD mice used in the experiments described herein is Taconic Farms (Germantown, NY). A large body of data, particularly from studies of the BB rat and NOD mouse has indicated that type I diabetes may be a T-cell mediated disease. Evidence to date suggests an important role for both major T cell subpopulations (CD4/L3T4 and CD8/Ly2) in the development of diabetes in man and in the NOD mouse. The data supporting the essential role of T cells in diabetes do not exclude the possibility that T lymphocytes may recruit other cells (e.g., macrophages) as the final effectors for β cell destruction. Macrophages have been implicated in the disease process based on their presence in the infiltrated islet and the ability of chronic silica treatment to prevent disease (see, e.g., Hutchings et al., 1990 [33] and references cited therein).

Using the NOD strain of mice, investigators have developed an acute transfer model of disease which

parall ls the sp ntaneous disease mod l in that transferred cells derived from diab t genic NOD mice m diat th disease process, which is characteriz d by immune reactive cells that mediate insulitis and islet β cell-specific destruction. Moreover, in this model, certain monoclonal antibodies against T cells (see, e.g., Miller et al., 1988 [5]) and macrophages (see, e.g., Hutchings et al., 1990 [33] have been shown to abrogate disease onset. Such monoclonal antibodies have been used in the treatment of spontaneous disease and adoptively transferred disease, for example, anti-CD4 antibody has been shown to abrogate disease in both models (Miller et al, 1988 [5] and Shizuru et al., 1988 [30]). Results of treatment with an agent in the adoptive transfer model or spontaneous disease model are indicative of the ability of the agent to modulate the human disease process.

EXAMPLE 1

Effect of Anti-VLA4 Antibody Treatment on Adoptive Transfer of Diabetes

For the adoptive transfer of diabetes experiments, NOD mice were obtained from Taconic Farms (Germantown, NY) or from the Joslin Diabetes Center (Boston, MA). Spontaneously diabetic (D) females of recent onset (13-20 weeks of age) were used as spleen cell donors and 8 week old nondiabetic (Y) females served as recipients. Spleen cells from 4 week old nondiabetic (Y) female donors which fail to transfer disease were used as a negative control.

Recipient mice were placed on acidified water (1:8400 dilution of concentrated HCl in water) one week prior to sublethal irradiation (775 rad) performed in split doses (300 rad, 300 rad, and 175 rad) on each of three days (day -2, -1, and the day of transfer), in order to minimize any incidence of intestinal infection subsequent to high dose irradiation (Gamma Cell 1000 Cesium ^{137} source, Nordion International, Inc., Ontario, Canada). Spleens were harvested from diabetic donors or from nondiabetic controls, cell suspensions made and red cells lysed with Hemolytic Geys solution. Spleen cells were injected intravenously (2-3 x 10^7 in 0.2 ml PBS) pretreated with either 75 μg R1-2 monoclonal antibody (mAb), 75 μg rat IgG2b, or untreated. For the antibody treatment, cells were simply suspended at 1-1.5 X 10^8 cells/ml with mAb at 375 μg/ml and kept on ice until injection. The timing of injection was within 3 hours after last irradiation. Some recipients received PBS alone. The anti-VLA4 mAb R1-2 and isotype-matched rat IgG2b were purchased from Pharmingen (La Jolla, CA). The R1-2 (rat anti-mouse) anti-VLA4 mAb was originally described by Holzmann et

al., 1989 [53]. The R1-2 anti-VLA4 mAb blocks VLA4 binding to its ligands (H ssion et al., 1992 [54]) and ther for belongs by definition to the B group (Pulido et al., 1991 [44], i.e., is equivalent to anti-human VLA4 mAbs of the B group (e.g., HP1/2 or HP2/1).

The R1-2 mAb or rat IgG2b was administered at a dose of 75 μg/0.2 ml intraperitoneally every 2-3 days, a dosing regimen which was determined to maintain maximal coating of VLA4-positive cells in the peripheral blood, lymphoid organs and bone marrow as detected by staining of peripheral blood cells and single cell suspensions prepared from these organs with a fluorochrome labelled mAb specific for the R1-2 mAb and FACS analysis to measure fluorochrome positive cells (as described above). Injections were maintained through day 12 or day 24 post transfer. Mice were monitored for diabetes by testing for glycosuria with TesTape (Eli Lilly, Indianapolis, IN) and by plasma glucose levels (Glucometer, 3 Blood Glucose Meter, Miles, Inc., Elkhart, IN) and were considered diabetic after two consecutive urine positive tests [Testape values of [+1] or higher] or plasma glucose levels >250 mg/dL.

An inhibitory effect of the anti-VLA4 mAb on the onset of diabetes was demonstrated when spleen cells isolated from NOD diabetic donors were treated with a saturating quantity of anti-VLA4 mAb R1-2 followed by transfer into nondiabetic irradiated hosts, as described above, and the R1-2 mAb was then administered every other day for 12 days in order to maintain maximal coating of all VLA4-positive cells in the peripheral blood and lymphoid organs for two weeks. Figure 1 shows the frequency of recipients that became diabetic and the day of disease onset for transfer of $2x10^7$ splenocytes from diabetic NOD donor (D→Y) (i)

without tr atment (closed circles); (ii) with rat IgG2b tr atment (closed triangles), and (iii) with R1-2 anti-VLA4 tr atment (closed diamonds) as well as for transfer of splenocytes from non-diabetic NOD donors (Y→Y) (open squares). Injection of PBS alone gave 0% incidence. Under these conditions, only 1 of 8 individual R1-2 mAb treated recipients became diabetic, with onset on day 29 post transfer. By contrast, 6/10 and 5/9 individuals became diabetic after receiving splenocytes from diabetic donors treated with no mAb or with non-specific rat IgG2b, respectively. As shown in Figure 1, diabetes onset occurred as early as day 14 post transfer, though administration of the irrelevant rat IgG2b somewhat delayed onset.

These data demonstrate a protective effect of the R1-2 mAb which was dependent upon its specificity for VLA4. Recipients of splenocytes from nondiabetic mice or of PBS alone failed to become diabetic. Thus, treatment with anti-VLA4 antibody reduced the frequency of diabetes during 30 days post transfer.

Although the results shown in Figure 1 demonstrate that clinical diabetes occurred in only 1 of 8 anti-VLA4 treated animals, it was possible that the anti-VLA4 antibody caused only a minor delay in the onset of disease. Plasma glucose levels were monitored in parallel with urine glucose in order to quantify any increase in blood sugar levels and thereby detect progression to clinical disease. In the anti-VLA4 antibody treated group shown in Figure 1, all mice were still normoglycemic on day 29 with an average plasma glucose value of 100 ± 7 mg/dL, n=7, except for the single individual who scored as clinically diabetic by urine test and plasma glucose >500 mg/dL. Thus, disease progression was not apparent in any of the other anti-VLA4 antibody treated recipients shown in

Figure 1 on day 29 post transfer, a full 2 weeks beyond the last anti-VLA4 antibody inj ction. Analysis of s ra from th se mic confirmed that the anti-VLA4 mAb dropped to low or undetectable levels by day 18-21 post-transfer.

Additional cell transfers were performed in order to confirm that the anti-VLA4 mAb protected against transfer of diabetes. In these experiments, the anti-VLA4 antibody treatment was extended to day 25 post transfer but administered every 3.5 days thereby maintaining saturating levels of R1-2 mAb or rat IgG2b through day 26 when mice were sacrificed for pancreatic tissue. Under these conditions, an inhibitory effect of the anti-VLA4 mAb on the onset of diabetes was also demonstrated upon spleen cell transfer and R1-2 treatment. Figure 2 shows the frequency of recipients (n=4-5 for each group) that became diabetic and the day of disease onset for transfer of $3x10^7$ splenocytes from diabetic NOD donors (D→Y) (i) without treatment (closed circles), (ii) with IgG2b treatment (closed triangles) and with R1-2 anti-VLA4 treatment (closed diamonds), as well as for transfer of splenocytes from non-diabetic NOD donors (Y→Y; open squares). Injection of PBS alone gave 0% incidence. Figure 2 shows that only 1 out of 5 R1-2 mAb treated mice became diabetic by day 22 post transfer whereas diabetes was transferred in 4/4 recipients without R1-2 mAb and 5/5 treated with rat IgG2b. Disease onset occurred as early as day 13 post transfer. These experiments, individually and collectively demonstrate that anti-VLA4 mAb reproducibly protects against development of diabetes in an acute transfer model of disease.

Further experiments were performed to determine whether the anti-VLA-4 mAb simply delayed disease onset during the treatment period or if it could achieve a

longer-term protective effect. Figure 3 shows th ons t of diabetes in mice over time aft r R1-2 injection (once every 3.5 days through day 25) with only 2/5 mice becoming diabetic on days 35 and 38 post transfer, 10-13 days after the last R1-2 injection. By contrast, diabetes occurred in the untreated and IgG2b treated groups as early as day 11 post transfer, with 100% incidence by days 18-21. Surprisingly, disease incidence in the R1-2 treated group did not further increase even as long as 2 months following the last R1-2 injection. Plasma glucose values monitored in parallel during this time reveal that these three individuals were consistently normoglycemic. After this point (i.e., approximately 3 months post-transfer), even the negative control groups which received PBS alone or non-diabetic cells begin developing spontaneous disease. In summary, the VLA-4-specific mAb reduces the incidence of diabetes transfer. Moreover, its protective effect against disease is sustained in the absence of further mAb treatment.

EXAMPLE 2

Effect of Anti-VLA4 mAb on Pancreatis Insulitis

For histological analysis, mice were sacrificed between 2-4 weeks post-transfer as described in this Example and pancreata harvested in 10% formalin buffered saline for paraffin-embedded sections which were stained with hematoxylin and eosin (H&E) for histology. Degree of insulitis was scored as follows: Grade 0: no insulitis [islet devoid of inflammation]; Grade I: peri-insulitis [inflammatory mononuclear cells located peripheral to the islet]; Grade II: <25% infiltrated [<25% of the islet interior contains lymphocytic inflammatory cells]; Grade III: 25-50% infiltrated [lymphocytic infiltration]; Grade IV: >50% infiltrated. The percent of islets in each Grade was then calculated relative to the total number of islets examined. Histologic sections were examined and scored for the degree of insulitis following the adoptive transfer of NOD splenocytes with and without anti-VLA4 mAb treatment and the results tabulated. Specifically, the frequency of uninfiltrated islets (Grade 0-I infiltrate) and islets with Grade II-IV insulitis (as described above) were quantitated. For each experimental group, pancreatic sections from n= 4-5 mice were scored.

Pancreatic tissue was recovered from recipients treated with the anti-VLA-4 mAb for various time periods in order to address its effect on the establishment of islet-specific cellular infiltrates. Mice were treated with nonspecific rat IgG2b or R1-2 mAb every 3.5 days through day 14 when sacrificed. Similarly, mice were treated through day 25 and sacrificed after diabetes was diagnosed or on day 26 post transfer. Mice continuously treated with the R1-2 mAb for 14 days post transfer maintain a high frequency

(76%) of uninfiltrated islets, with only 24% progr ssing to grade II-IV insulitis. By contrast those tr ated with nonspecific rat IgG2b show the reciprocal pattern, with 74% severe insulitis. Likewise, in the mice treated with R1-2 though day 25 (20% diabetic, pancreata isolated from mice reported in Figure 2), a high frequency (58%) of uninfiltrated islets were preserved, similar to that (55% uninfiltrated) in nondiabetic recipients of young NOD splenocytes, as shown in Figure 4. By contrast, both the untreated or IgG2b-treated mice had only 28% uninfiltrated islets, and conversely had increased (72%) insulitis. Thus, the anti-VLA-4 mAb treatment appears to specifically inhibit or alternatively to delay the development of insulitis upon adoptive transfer of diabetogenic spleen cells.

In order to distinguish between these alternatives, the pattern of insulitis after 4 weeks post transfer was determined when mice were treated with rat IgG2b or R1-2 mAb through day 12 and then maintained without further treatment. Mice were sacrificed upon diabetes diagnosis or on day 29 post transfer. Analysis of sera from these mice confirmed that circulating anti-VLA-4 mAb dropped to undetectable levels by days 18-21 post transfer. With this protocol, the degree of insulitis in the R1-2-treated group (69% insulitis, 25% diabetic) was similar to that in untreated recipients (73% insulitis, 60% diabetic) though still lower than that in the rat IgG2b-treated mice (96% insulitis, 75% diabetic), as shown in Figure 5. Significantly, the severity of insulitis was similar between the R1-2 treated, untreated and rat IgG2b treated groups with an average of 57%, 47%, 64% Grade III/IV infiltrates, respectively. Even considering only the nondiabetic R1-2 treated

individuals, they still exhibited 59% insulitis with 52% Grade III/IV infiltrates. Recipients of nondiabetogenic NOD splenocytes had only 7% Grade III/IV infiltrates. Conversely, Figure 5 shows that the frequency of uninfiltrated islets was decreased in the R1-2 treated mice as compared to recipients of saline or nondiabetogenic spleen cells. Thus, the degree of insulitis progressed in these R1-2 treated mice (Figure 5) as compared to mice wherein R1-2 treatment was maintained (Figure 4) and approached that in the untreated and rat IgG2b treated control groups. Taken together, these data indicate that anti-VLA-4 mAb administration can delay the progression of insulitis in an acute transfer model of disease.

EXAMPLE 3

Comparison of Different Anti-VLA4 Antibody Treatment in Adoptive Transfer of Diabetes

This Example provides comparative efficacy results of PS/2, an anti-VLA4 antibody, with R1-2 using the adoptive transfer model and procedure described in Example 1. NOD mice were treated with (a) an irrelevant control antibody (D/rat IgG2b, n = 19 mice); (b) R1-2 antibody (D/R1-2 mAb, n = 24 mice); (c) PS/2 mAb (D/PS/2 mAb, n = 5 mice); or (d) no treatment (NONE, n = 26 mice). Spleen cells were injected intravenously (2-3x 10^7 in 0.2 ml PBS) and pretreated with either 75 μg R1-2 mAb, 75 μg PS/2 mAb, 75 μg rat IgG2b, or untreated. Isolation and purification of PS/2 anti-VLA4 mAb was originally described by Miyake et al., 1991 [55].

The R1-2 mAb, PS/2 mAb or rat IgG2b was administered at a dose of 75 μg/0.2 ml intraperitoneally every 2-3 days, a dosing regimen which was determined to maintain maximal coating of VLA4-positive cells in the peripheral blood, lymphoid organs and bone marrow as detected by staining of peripheral blood cells and single cell suspensions prepared from these organs with a fluorochrome labelled mAb specific for the R1-2 and PS/2 mAb and FACS analysis to measure fluorochrome positive cells (as described above). Injections were maintained through days 22 to 25 post transfer. Mice were monitored for diabetes by testing for glycosuria with TesTape (Eli Lilly, Indianapolis, IN) and by plasma glucose levels (Glucometer, 3 Blood Glucose Meter, Miles, Inc., Elkhart, IN) and were considered diabetic after two consecutive urine positive tests [Testape values of [+1] or higher] or plasma glucose levels >250 mg/dL.

An inhibitory effect of the anti-VLA4 mAb on the onset of diabetes was demonstrated when spleen cells isolated from NOD diabetic donors were treated with a saturating quantity of anti-VLA4 mAb R1-2 or PS/2 followed by transfer into nondiabetic irradiated hosts, as described above, and the R1-2 mAb or PS/2 mAb was then administered every other day for 22-25 days in order to maintain maximal coating of all VLA4-positive cells in the peripheral blood and lymphoid organs for about two weeks. Table 1 shows the frequency of recipients that became diabetic and the day of disease onset for transfer of splenocytes from diabetic NOD donor (i) without treatment (D); (ii) with rat IgG2b treatment (D/nonspecific rat IgG2b); (iii) with R1-2 anti-VLA4 treatment (D/R1-2 mAb); (iv) with PS/2 treatment (D/PS/2 mAb) as well as for transfer of splenocytes from non-diabetic NOD donors (non-D). Non-diabetic mice receiving PBS and no splenocytes (NONE) were included as a control. Injection of PBS alone gave 4% incidence. Under these conditions, only 1 of 24 individual R1-2 mAb treated recipients became diabetic, with onset on day 22 post transfer while none of the five individual PS/2 mAb treated recipients became diabetic. By contrast, 16/19 individuals became diabetic after receiving splenocytes from diabetic donors treated with no mAb or with non-specific rat IgG2b. As shown in Table 1, diabetes onset occurred as early as day 14 post transfer, though administration of the irrelevant rat IgG2b somewhat delayed onset by one day.

These data demonstrate a protective effect of the R1-2 mAb and PS/2 which were dependent upon its specificity for VLA4. Recipients of splenocytes from nondiabetic mice or of PBS alone failed to become diabetic. Thus, treatment with anti-VLA4 antibody

r duced the frequency of diabet s during 30 days post transfer. Analysis of sera from these mic confirmed that l vels of R1-2 and PS/2 anti-VLA4 mAb become undetectable between days 26 and 34 post-transfer.

TABLE 1

Anti-VLA-4 mAbs Inhibit Adoptive Transfer of Diabetes in NOD Mice

Cells Transferred/Treatment*	No. Diabetic/Total Recipients+		Day of Onset X ± SEM
NONE	1/26	(4%)	34
Non-D	1/15	(7%)	15
D	16/19	(84%)	14 ± 0.2
D/Nonspecific rat IgG2b	16/19	(84%)	15 ± 0.9
D/R1-2 mAb	1/24	(4%)	22
D/PS/2 mAb	0/5	(0%)	

*Spleen cells from 4 week old nondiabetic (NON-D) or from new onset diabetic (D) NOD females were transferred, with D cells suspended in mAb or rat IgG or without mAb before transfer and recipients treated twice weekly for 22-25 days. Mice were monitored for one month post transfer. Data are compiled from 5 experiments.

+D/R1-2 and D/PS/2 mAb treated groups are significantly different from D and D/rat IgG2b treated groups by Chi square test with Yates' correction as follows: R1-2 vs. IgG2b treated and D group, $p<0.0001$; PS/2 vs. IgG2b treated and D group, $p<0.003$.

EXAMPLE 4

Effect of Anti-VLA4 Antibody Treatment on Spontaneous Diabetes Model

This Example described efficacy results using R1-2 mAb in the spontaneous diabetes model which employs NOD mice. NOD mice were treated for 8 weeks with (a) an irrelevant control antibody (NOD/rat IgG2b, n = 10 mice); (b) R1-2 antibody (NOD/R1-2, n = 20 mice); or (c) no treatment (NOD, n = 10 mice) starting at week four to week twelve of age. mAb was administered at a dose of 75 μg in 0.2 ml PBS iv, twice weekly. Mice were monitored for diabetic events by TesTape for glycosuria as previously described.

Figure 6 demonstrates a marked delay in diabetes onset (12-16 weeks delay) following R1-2 administration, as compared to the two control groups. NOD mice which received irrelevant IgG2b mAb or no treatment developed diabetes as early as 13 weeks. These spontaneous disease model results parallel the adoptive transfer results with R1-2 mAb illustrated in Figure 1 and directly demonstrate that an anti-VlA4 antibody protects against diabetes onset.

EXAMPLE 5

Effect of a VCAM-Ig Fusion Protein on Adoptive Transfer of Diabetes

The adoptive transfer experiment described in Example 1 was repeated with a VCAM-Ig fusion protein (VCAM 2D-IgG) instead of an anti-VLA4 mAb. VCAM 2D-IgG is a soluble form of the ligand for VLA4 (VCAM1) which consists of the two N-terminal domains of VCAM1 fused to the human IgG1 heavy chain constant region sequences (Hinges, C_H2 and C_H3). The VCAM 2D-IgG DNA sequence and its translated amino acid sequence are shown in SEQ ID NO: 9. Figure 8 illustrates the fusion protein structure. The fusion protein was constructed by recombinant techniques as described below.

Isolation of cDNA of Human IgG1 Heavy Chain Region and Construction of Plasmid pSAB144

In order to isolate a cDNA copy of the human IgG1 heavy chain region, RNA was prepared from COS7 cells which has been transiently transfected by the plasmid VCAM1-IgG1 (also known as pSAB133). Construction of plasmid VCAM1-IgG1 is described in PCT patent application WO 90/13300. The RNA was reverse transcribed to generate cDNA using reverse transcriptase and random hexamers as the primers. After 30 min. at 42°C, the reverse transcriptase reaction was terminated by incubation of the reaction at 95°C for 5 min. The cDNA was then amplified by PCR (Polymerase Chain Reaction, see, e.g., Sambrook et al., Molecular Cloning, Vol. 3, pp. 14.1-14.35 (Cold Spring Harbor; 1989)) using the following kinased primers: 370-31 (SEQ ID NO: 10):

5'TCGTC GAC AAA ACT CAC ACA TGC C
Asp Lys Thr His Thr Cys

which contains a SalI site, and

370-32 (SEQ ID NO: 11):

5' GTAAATGAGT GCGGCGGCCG CCAA,

which encodes the carboxy terminal lysine of the IgG1 heavy chain constant region, followed by a NotI site.

The PCR amplified cDNA was purified by agarose gel electrophoresis and glass bead elution for cloning in plasmid pNN03. Plasmid pNN03 was constructed by removing the synthetic polylinker sequence from the commercially available plasmid pUC8 (Pharmacia, Piscataway, New Jersey) by restriction endonuclease digestion and replacing the synthetic polylinker sequence with the following novel synthetic sequence (SEQ ID NO: 12):

GCGGCCGCGG TCCAACCACC AATCTCAAAG CTTGGTACCC GGGAATTCAG ATCTGCAGCA TGCTCGAGCT CTAGATATCG ATTCCATGGA TCCTCACATC CCAATCCGCG GCCGC.

The purified PCR amplified cDNA fragment was ligated to pNN03 which had been cleaved with EcoRV, dephosphorylated, and purified by low melt agarose gel electrophoresis. The ligation reaction was used to transform E.coli JA221 and the resulting colonies were screened for a plasmid containing an insert of approximately 700 bp. The identity of the correct insert was confirmed by DNA sequence analysis, and the plasmid was designated pSAB144.

Construction of Plasmid pSAB142

The plasmid pSAB142 was constructed as follows. CDNA prepared from COS cells transfected with pSAB133 (as described in the previous section) was subjected to PCR amplification using obligonucleotides 370-01 and 370-29. Oligonucleotide 370-01 includes a NotI site and the nucleotides corresponding to amino acids 1 through 7 of the VCAM-1 signal sequence

(SEQ ID NO: 13):

5' GAGCTCGAGGCGGCCGCACCATGCCTGGGAAGATGGTCGTG
MetProGlyLysMetValVal

Oligonucleotide 370-29 corresponds to the VCAM-1 amino acids 214-219 and includes a SalI site (SEQ ID NO: 14):

5'AA GTC GAC TTG CAA TTC TTT TAC

The amplified DNA fragment was ligated to the vector fragment of pNN03, cleaved by EcoRV.

Construction of pSAB132

pJOD-S (Barsoum, J., DNA and Cell Biol., 9, pp.293-300 (1990)) was modified to insert a unique NotI site downstream from the adenovirus major late promoter so that NotI fragments could be inserted into the expression vector. pJOD-S was linearized by NotI cleavage of the plasmid DNA. The protruding 5' termini were blunt-ended using Mung Bean nuclease, and the linearized DNA fragment was purified by low melting temperature agarose gel electrophoresis. The DNA fragment was religated using T4 DNA ligase. The ligated molecules were then transformed into E.coli JA221. Colonies were screened for the absence of a NotI site. The resulting vector was designated pJOD-S delta Not1. pJOD-8 delta Not1 was linearized using SalI and the 5' termini were dephosphorylated using calf alkaline phosphatase. The linearized DNA fragment was purified by low melting temperature agarose gel eletrophoresis and ligated in the presence of phosphorylated oligonucleotide ACE175, which has the following sequence (SEQ ID NO:15):

TCGACGCGGC CGCG

The ligation mixture was transformed into E.coli JA221, and colonies were screened for the presence of a

plasmid having a NotI site. The desired plasmid was named pMDR901.

In order to delete the two SV40 enhancer repeats in the Sv40 promoter which controls transcription of the DHFR cDNA, pMDR901 and pJODΔe-tPA (Barsoum, DNA and Cell Biol., 9, pp. 293-300 (1990)), both were cleaved with AatII and DraIII. The 2578 bp AatII-DraIII fragment from pMDR901 and the 5424 bp AatII-DraIII fragment from pJODΔe-tPA were isolated by low melting temperature agarose gel electrophoresis and ligated together. Following transformation into E.coli JA221, the resulting plasmid, pMDR902, was isolated. pSAB132 was then formed by eliminating the EcoRI-NotI fragment of pMDR902 containing the adenovirus major late promoter and replacing it with an 839 bp EcoRI-NotI fragment from plasmid pCMV-B (Clontech, Palo Alto, California) containing the human cytomegalovirus immediate early promoter and enhancer.

Construction of pSAB146

pSAB144 was cleaved with SalI and NotI, and the 693 bp fragment isolated. pSAB142 was cleaved with NotI and SalI and the 664 bp fragment was isolated. The two fragments were ligated to pSAB132 which had been cleaved with NotI, and the 5′ termini dephosphorylated by calf alkaline phosphatase. The resulting plasmid, pSAB146, contained the DNA sequence encoding the VCAM-1 signal sequence, the amino terminal 219 amino acids of mature VCAM-1, ten amino acids of the hinge region of IgG1 and the C_H2 and C_H3 constant domains of IgG1.

Production of VCAM 2D-IgG from a stably transformed CHO cell line

A recombinant VCAM 2D-IgG expression vector was constructed as described below and transfected into CHO cells to produce a cell line continuously secreting VCAM 2D-IgG.

The 1.357 kb NotI fragment containing the VCAM 2D-IgG coding sequence of pSAB146 was purified by agarose gel electrophoresis. This fragment was ligated into the NotI cloning site of the expression vector pMDR901, which uses the adenovirus 2 major late promoter for heterologous gene expression and the selectable, amplifiable dihydrofolate reductase (dhfr) marker. The ligated DNA was used to transform E.coli DH5. Colonies containing the plasmid with the desired, correctly oriented insert were identified by the presence of 5853 and 3734 bp fragments upon digestion with Hind III; and 4301, 2555, 2293, and 438 bp fragments upon digestion with BglII. The resultant recombinanat VCAM 2D-IgG expression vector was designated pEAG100. The identity of the correct insert was confirmed by DNA sequence analysis.

The recombinant expression plasmid pEAG100 was electroporated into dhfr-deficient CHO cells according to the published protocol of J. Barsoum (DNA Cell Biol 9: 293-300, 1990), with the following changes: 200 μg of PvuI-linearized pEAG100 plasmid and 200 μg of sonicated salmon sperm DNA were used in the electroporation protocol. In addition, cells were selected in alpha-complete medium supplemented with 200 nM methotrexate.

To determine expression levels of secreted VCAM 2D-IgG, clones were transferred to a flat bottom 96 well microtiter plate, grown to confluency and assayed by ELISA as described below.

Wells of Immulon 2 plates (Dynatech, Chantilly, Virginia) were each coated with anti-VCAM MAb 4B9 (isolat d and purifi d on Protein A Sephar s as described by Carlos et al, 1990 [56]) with 100µl of anti-VCAM 4B9 MAb diluted to 10µg/ml in 0.05 M sodium carbonate/bicarbonate buffer, pH 9.6, covered with Parafilm, and incubated overnight at 4°C. The next day, the plate contents were dumped out and blocked with 200µl/well of a block buffer (5% fetal calf serum in 1x PBS), which had been filtered through a 2µ filter. The buffer was removed after a 1 hour incubation at room temperature and the plates were washed twice with a solution of 0.05% Tween-20 in 1X PBS. Conditioned medium was added at various dilutions. As a positive control, an anti-mouse Ig was also included. Block buffer and LFA-3TIP constituted as negative controls. The samples and controls were incubated at room temperature for 2 hours.

The plates were then washed twice with a solution of 0.05% Tween-20 in 1X PBS. Each well, except for the positive control well, was then filled with 50µl of a 1:2000 dilution of HRP-Donkey anti-human IgG (H+L) (Jackson Immune Research Laboratories, Inc.; West Grove, Pennsylvania) in block buffer. The positive control well was filled with 50 µl of a 1:2000 dilution of HRP-Goat anti-mouse lgG (H+L)(Jackson Immune Research Laboratories, Inc.; West Grove, Pennsylvania) in block buffer. The plates were then incubated for 1 hour at room temperature.

The HRP conjugated Ab solutions were removed, and the wells were washed twice with 0.05% Tween-20 in 1X PBS. Then, 100 µl of HRP-substrate buffer was added to each well at room temperature. HRP-substrate buffer was prepared as follows: 0.5 ml of 42mM 3,3', 5,5'-tetramethylbenzidine (TMB), (ICN Immunobiologicals,

Lisle, South Carolina, Catalogue No. 980501) in DMSO (Aldrich) was slowly added to 50 ml of substrate buffer (0.1 M sodium acetate/citric acid, pH4.9); follow d by addition of 7.5 µl of 30% hydrogen peroxide (Sigma, Catalogue No. H-1009).

The development of a blue color in each well was monitored at 650nm on a microtiter plate reader. After 7-10 minutes, the development was stopped by the addition of 100 µl of 2N Sulfuric acid. The resulting yellow color was read at 450nm on a microtiter plate reader. A negative control well was used to blank the machine.

Purification of VCAM 2D-IgG

CHO cells expressing VCAM 2D-IgG were grown in roller bottles on collagen beads. Conditioned medium (5 Liters) was concentrated to 500 ml using an Amicon S1Y10 spiral ultrafiltration cartridge (Amicon, Danvers, MA). The concentrate was diluted with 1 liter of Pierce Protein A binding buffer (Pierce, Rockford, IL) and gravity loaded onto a 10 ml Protein A column (Sepharose 4 Fast Flow, Pharmacia, Piscataway, NJ). The column was washed 9 times with 10 ml of Protein A binding buffer and then 7 times with 10 ml of PBS. VCAM 2D-IgG was eluted with twelve-5 ml steps containing 25 mM H_3PO_4 pH2.8, 100 mM NaCl. The eluted samples were neutralized by adding 0.5 M Na_2HPO_4 pH8.6 to 25 mM. Fractions were analyzed for absorbance at 280 nm and by SDS-PAGE. The three peaks fractions of highest purity were pooled, filtered, aliquoted and stored at -70°C. By SDS-PAGE, the product was greater than 95% pure. The material contained less than 1 endotoxin unit per mg of protein. In some instances, it was necessary to further purify the Protein A eluate product on Q-Sepharose FF (Pharmacia). The protein A

luate was diluted with 3 volumes of 25 mM Tris HCl pH 8.0 and load d onto a Q-Sepharose FF column at 10 mg VCAM 2D-IgG per ml f r sin. The VCAM 2D-IgG was then eluted from the Q- Sepharose with PBS.

Evaluation of VCAM 2D-IgG

Spleen cell suspensions were prepared from diabetic donors or from nondiabetic controls as described above. Spleen cells were injected intraveneously (2-3 x 10^7 in 0.2 ml PBS) and were pretreated with either 100μg VCAM 2D-IgG or 100μg of irrelevant LFA-3Ig fusion protein control. Another group received PBS alone without cells transferred. The fusion protein LFA-3Ig (LFA-3TIP) was isolated and purified as described in PCT US92/02050 and Miller et al., 1993 [57]. The VCAM 2D-IgG fusion protein or irrelevant LFA-3Ig protein was administered at a dose of 100 μg/0.2 ml intraperitoneally twice weekly through day 17. This concentration was sufficient to provide a serum level of fusion protein sufficient to saturate VLA4-positive cells, the serum levels determined by ELISA as described above. Diabetes onset was monitored as described above.

The results of the evaluation are shown in Figure 7. As shown in this Figure, VCAM 2D-IgG fusion protein significantly inhibits the onset of diabetes in recipients of cells from diabetic donor mice (D/VCAM-Ig, open circles) with 60% incidence by day 30 post-transfer, as compared to the mice which received cells from diabetic donor (data not shown) and LFA-3Ig irrelevant control Ig fusion protein (D/LFA-3 Ig) which had already achieved 60% incidence by day 15 post-transfer. Mice which received no cells (PBS only) did not develop disease. There were n = 5 mice per experimental group.

In summary, VLA4 binding agents such as anti-VLA4 antibodies were protective against diabetes disease onset (Examples 1, 3 and 4) and were effective in delaying the progression of insulitis (Example 2) using a murine model for human diabetes. Other VLA4 binding agents such as soluble VCAM derivatives (VCAM 2D-IgG) were also useful in protecting against diabetes disease onset (Example 5). The foregoing examples are intended as an illustration of the method of the present invention and are not presented as a limitation of the invention as claimed hereinafter. From the foregoing disclosure, numerous modifications and additional embodiments of the invention will be apparent to those experienced in this art. For example, actual dosage used, the type of antibody or antibody fragment used, mode of administration, exact composition, time and manner of administration of the treatment, and many other features all may be varied without departing from the description above. All such modifications and additional embodiments are within the contemplation of this application and within the scope of the appended claims.

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The foregoing documents are incorporated herein by reference in their entirety.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Burkly, Linda C.

(ii) TITLE OF INVENTION: Treatment for Insulin Dependent Diabetes

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Allegretti & Witcoff, Ltd.
(B) STREET: 10 South Wacker Drive, Suite 3000
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: US
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Word Perfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/029,330
(B) FILING DATE: 9 February 1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Miao, Emily
(B) REGISTRATION NUMBER: 35,285
(C) REFERENCE/DOCKET NUMBER: 92,749-A; D015 CIP PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312-715-1000
(B) TELEFAX: 312-715-1234

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

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(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "pBAG159 insert: HP1/2 h avy chaih variable regi n; amino acid 1 is Glu (E) but Gln (Q) may be substituted"

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(B) LOCATION: 1-360

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                   21                  26                  31

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Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu
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Asn Thr Ala Trp Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
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GCC GTC TAC TAC TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT 315
Ala Val Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr
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GCT CTG GAC TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA 360
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(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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                   36                  41                  46

Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
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Pro Lys Phe Gln Val Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser
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Asn Thr Ala Trp Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(A) NAME/KEY: CDS
(B) LOCATION: 1-318
(D) OTHER INFORMATION: /product= "HP1/2 light chain variable region"

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(B) LOCATION: 1
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ATC                                                         318
Ile
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(D) TOPOLOGY: linear

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Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
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                50                  55                  60

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Ile
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(D) TOPOLOGY: linear

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(B) LOCATION: 1-57

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(A) NAME/KEY: mat_peptide
(B) LOCATION: 58-429

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1-429

(ix) FEATURE:
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15 20 25

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(B) LOCATION: 1-57

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 58-386

(ix) EATURE:
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(B) LOCATION: 1-386

(ix) FEATURE:
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC  45
M t Gly Trp Ser Cys Ile Il  Leu Phe Leu Val Ala Thr Ala Thr
               -15                 -10                 -5

GGT GTC CAC TCC AGC ATC GTG ATG ACC CAG AGC CCA AGC AGC CTG  90
Gly Val His Ser Ser Ile Val Met Thr Gln Ser Pro Ser Ser Leu
            1                   5                  10

AGC GCC AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT 135
Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser
            15                  20                  25

CAG AGT GTG ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT 180

Gln Ser Val Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly
            30                  35                  40

AAG GCT CCA AAG CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT 225
Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr
            45                  50                  55

GGT GTG CCA GAT AGA TTC AGC GGT AGC GGT TAT GGT ACC GAC TTC 270
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe
            60                  65                  70

ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC 315
Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr
            75                  80                  85

TAC TGC CAG CAG GAT TAT AGC TCT CCG TAC ACG TTC GGC CAA GGG 360
Tyr Cys Gln Gln Asp Tyr Ser Ser Pro Tyr Thr Phe Gly Gln Gly
            90                  95                 100

ACC AAG GTG GAA ATC AAA CGT AAG TG                          386
Thr Lys Val Glu Ile Lys Arg Lys
           105
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
               -15                 -10                 -5
```

Gly Val His Ser Ser Ile Val Met Thr Gln S r Pro Ser Ser Leu
1 5 10

S r Ala Ser Val Gly Asp Arg Val Thr Il Thr Cys Lys Ala Ser
15 20 25

Gln Ser Val Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly
30 35 40

Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr
45 50 55

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe
60 65 70

Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr
75 80 85

Tyr Cys Gln Gln Asp Tyr Ser Ser Pro Tyr Thr Phe Gly Gln Gly
90 95 100

Thr Lys Val Glu Ile Lys Arg Lys
105

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1348 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: VCAM-1 gene segment
(B) LOCATION: 1-219
(D) OTHER INFORMATION: This portion of the sequence corresponds, in part, to Exons I, II and III nucleotide sequence of the VCAM-1 gene of Cybulsky et al. Proc. Nat'l. Acad. Sci. USA 88:7861 (1991).

(ix) FEATURE:
(A) NAME/KEY: Hinge region
(B) LOCATION: 220-229
(D) OTHER INFORMATION: This portion of the sequence corresponds, in part, to Fig. 12A in PCT/US92/0250 and represents the hinge region of Human IgG1 heavy chain constant region.

(ix) FEATURE:
(A) NAME/KEY: Heavy chain constant region 2
(B) LOCATION: 230-338
(D) OTHER INFORMATION: This portion of the sequence corresponds, in part, to Fig. 12A in PCT/US92/02050 and represents the heavy chain constant region 2 of Human IgG1 heavy chain constant region.

(ix) FEATURE:
(A) NAME/KEY: heavy chain constant region 3
(B) LOCATION: 339-446
(D) OTHER INFORMATION: This portion of the sequence corresponds, in part, to Fig. 12A in PCT/US92/02050 and represents the heavy chain constant region 3 of Human IgG1 heavy chain constant region.

```
ATG CCT GGG AAG ATG GTC GTG ATC CTT GGA GCC TCA AAT ATA CTT   45
Met Pro Gly Lys Met Val Val Ile Leu Gly Ala Ser Asn Ile Leu
                5                  10                  15

TGG ATA ATG TTT GCA GCT TCT CAA GCT TTT AAA ATC GAG ACC ACC   90
Trp Ile Met Phe Ala Ala Ser Gln Ala Phe Lys Ile Glu Thr Thr
            20                  25                  30

CCA GAA TCT AGA TAT CTT GCT CAG ATT GGT GAC TCC GTC TCA TTG  135
Pro Glu Ser Arg Tyr Leu Ala Gln Ile Gly Asp Ser Val Ser Leu
        35                  40                  45

ACT TGC AGC ACC ACA GGC TGT GAG TCC CCA TTT TTC TCT TGG AGA  180
Thr Cys Ser Thr Thr Gly Cys Glu Ser Pro Phe Phe Ser Trp Arg
    50                  55                  60

ACC CAG ATA GAT AGT CCA CTG AAT GGG AAG GTG ACG AAT GAG GGG  225
Thr Gln Ile Asp Ser Pro Leu Asn Gly Lys Val Thr Asn Glu Gly
65                  70                  75

ACC ACA TCT ACG CTG ACA ATG AAT CCT GTT AGT TTT GGG AAC GAA  270
Thr Thr Ser Thr Leu Thr Met Asn Pro Val Ser Phe Gly Asn Glu
            80                  85                  90

CAC TCT TAC CTG TGC ACA GCA ACT TGT GAA TCT AGG AAA TTG GAA  315
His Ser Tyr Leu Cys Thr Ala Thr Cys Glu Ser Arg Lys Leu Glu
        95                  100                 105

AAA GGA ATC CAG GTG GAG ATC TAC TCT TTT CCT AAG GAT CCA GAG  360
Lys Gly Ile Gln Val Glu Ile Tyr Ser Phe Pro Lys Asp Pro Glu
    110                 115                 120

ATT CAT TTG AGT GGC CCT CTG GAG GCT GGG AAG CCG ATC ACA GTC  405
Ile His Leu Ser Gly Pro Leu Glu Ala Gly Lys Pro Ile Thr Val
```

```
                    125                 130                 135

AAG TGT TCA GTT GCT GAT GTA TAC CCA TTT GAC AGG CTG GAG ATA   450
Lys Cys Ser Val Ala Asp Val Tyr Pro Ph  Asp Arg Leu Glu Ile
                    140                 145                 150

GAC TTA CTG AAA GGA GAT CAT CTC ATG AAG AGT CAG GAA TTT CTG   495
Asp Leu Leu Lys Gly Asp His Leu Met Lys Ser Gln Glu Phe Leu
                    155                 160                 165

GAG GAT GCA GAC AGG AAG TCC CTG GAA ACC AAG AGT TTG GAA GTA   540
Glu Asp Ala Asp Arg Lys Ser Leu Glu Thr Lys Ser Leu Glu Val
                    170                 175                 180

ACC TTT ACT CCT GTC ATT GAG GAT ATT GGA AAA GTT CTT GTT TGC   585
Thr Phe Thr Pro Val Ile Glu Asp Ile Gly Lys Val Leu Val Cys
                    185                 190                 195

CGA GCT AAA TTA CAC ATT GAT GAA ATG GAT TCT GTG CCC ACA GTA   630
Arg Ala Lys Leu His Ile Asp Glu Met Asp Ser Val Pro Thr Val
                    200                 205                 210

AGG CAG GCT GTA AAA GAA TTG CAA GTC GAC AAA ACT CAC ACA TGC   675
Arg Gln Ala Val Lys Glu Leu Gln Val Asp Lys Thr His Thr Cys
                    215                 220                 225

CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC   720
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
                    230                 235                 240

CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC   765
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                    245                 250                 255

CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT   810
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                    260                 265                 270

GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT   855
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
                    275                 280                 285

GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGG   900
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                    290                 295                 300

GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC   945
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                    305                 310                 315

AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC   990
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
```

```
                  320                 325                 330

ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA 1035
Ile Glu Lys Thr Il  Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                  335                 340                 345

CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC 1080
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
                  350                 355                 360

CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC 1125
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
                  365                 370                 375

ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC 1170
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                  380                 385                 390

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC 1215
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                  395                 400                 405

TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC 1260
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                  410                 415                 420

GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC 1305
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
                  425                 430                 435

ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA GTG CGG      1348
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                  440                 445
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION: This corresponds to Kinase Primer 370-31.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCGTC GAC AAA ACT CAC ACA TGC C 24
Asp Lys Thr His Thr Cys
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION: This corresponds to Kinase Primer 370-32.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAAATGAGT GCGGCGGCCG CCAA 24

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGGCCGCGG TCCAACCACC AATCTCAAAG CTTGGTACCC GGGAATTCAG 50

ATCTGCAGCA TGCTCGAGCT CTAGATATCG ATTCCATGGA TCCTCACATC 100

CCAATCCGCG GCCGC 115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GA GCT CGA GGC GGC CGC ACC ATG CCT GGG AAG ATG GTC GTG 41
Met Pro Gly Lys Met Val Val
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AA GTC GAC TTG CAA TTC TTT TAC 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY:

(B) LOCATION:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCGACGCGGC CGCG 14

CLAIMS:

1. A method for the prevention of insulin dependent (type I) diabetes comprising administering to a prediabetic individual a composition comprising an anti-VLA4 antibody.

2. A method according to claim 1, wherein the anti-VLA4 antibody selected from the group consisting of HP1/2, HP2/1, HP2/4, L25, and P4C2.

3. A method according to claim 1, wherein the anti-VLA4 antibody is HP1/2, or a fragment thereof, capable of binding to VLA4.

4. A method according to claim 1, wherein the anti-VLA4 antibody is a humanized HP1/2 antibody, or a fragment thereof, capable of binding to VLA4.

5. A method according to claim 1, wherein the composition is administered at a dosage so as to provide from about 0.1 to about 10 mg/kg, based on the weight of the prediabetic individual.

6. A method according to claim 1, wherein the composition is administered in an amount effective to coat VLA4-positive cells in the peripheral blood for a period of 1-14 days.

7. A method according to claim 1, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the prediabetic individual of at least 1 μg/ml.

8. A method according to claim 1, wherein the composition is administered prior to the development of overt diabetes, as measured by a serum glucose level of less than about 250 mg/dL.

9. A method according to claim 1, wherein the prediabetic individual is a human.

10. A method for the treatment of diabetes comprising administering to a mammal with a susceptibility to diabetes, an antibody, a recombinant antibody, a chimeric antibody, fragments of such antibodies, a polypeptide or a small

molecule capable of binding to the α_4 subunit of VLA4, or combinations of any of the foregoing, in an amount effective to provide inhibition of onset of diab tes.

11. A method according to claim 10, wherein the antibody, polypeptide or molecule is selected from monoclonal antibody HP1/2; Fab, Fab', $F(ab')_2$ or F(v) fragments of such antibody; soluble VCAM-1 or fibronectin polypeptides; or small molecules that bind to the VCAM-1 or fibronectin binding domain of VLA4.

12. A method according to claim 10, wherein the composition comprises a plurality of anti-VLA4 monoclonal antibodies or VLA4-binding fragments thereof.

13. A method according to claim 10, wherein the composition is administered at a dosage so as to provide from about 0.1 to about 10 mg/kg of antibody, antibody fragment, polypeptide or small molecule, based on the weight of the susceptible mammal.

14. A method according to claim 10, wherein the composition is administered in an amount effective to coat VLA4-positive cells in the peripheral blood for a period of 1-14 days.

15. A method according to claim 10, wherein the composition is administered in an amount effective to provide a plasma level of antibody in the mammal of at least 1 μg/ml over a period of 1-14 days.

16. A method according to Claim 11, wherein the soluble VCAM-1 polypeptides comprise VCAM 2D-IgG.

17. A pharmaceutical composition effective to provide inhibition of onset of diabetes consisting essentially of a monoclonal antibody recognizing VLA4 in a pharmaceutically acceptable carrier.

FIG. 1

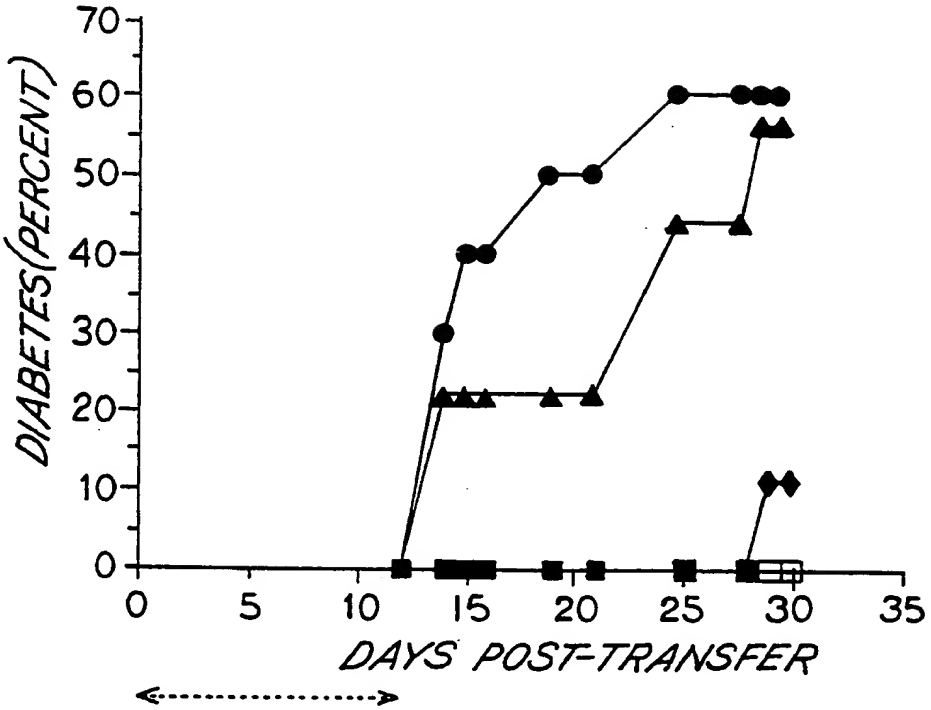

DIABETES (PERCENT)
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
DAYS POST-TRANSFER

FIG. 2

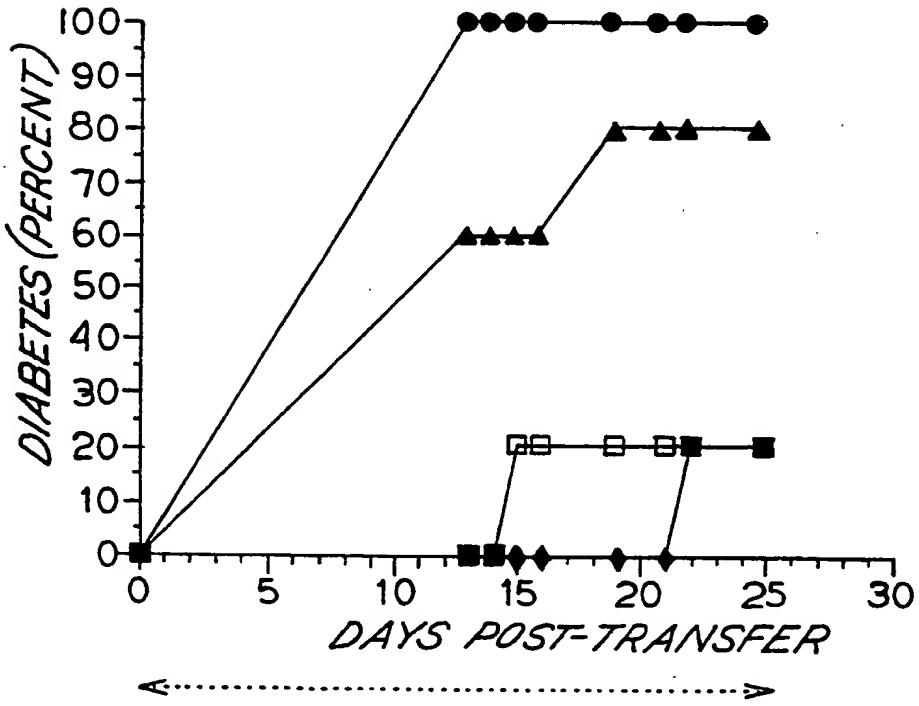

DIABETES (PERCENT)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
DAYS POST-TRANSFER

FIG. 3

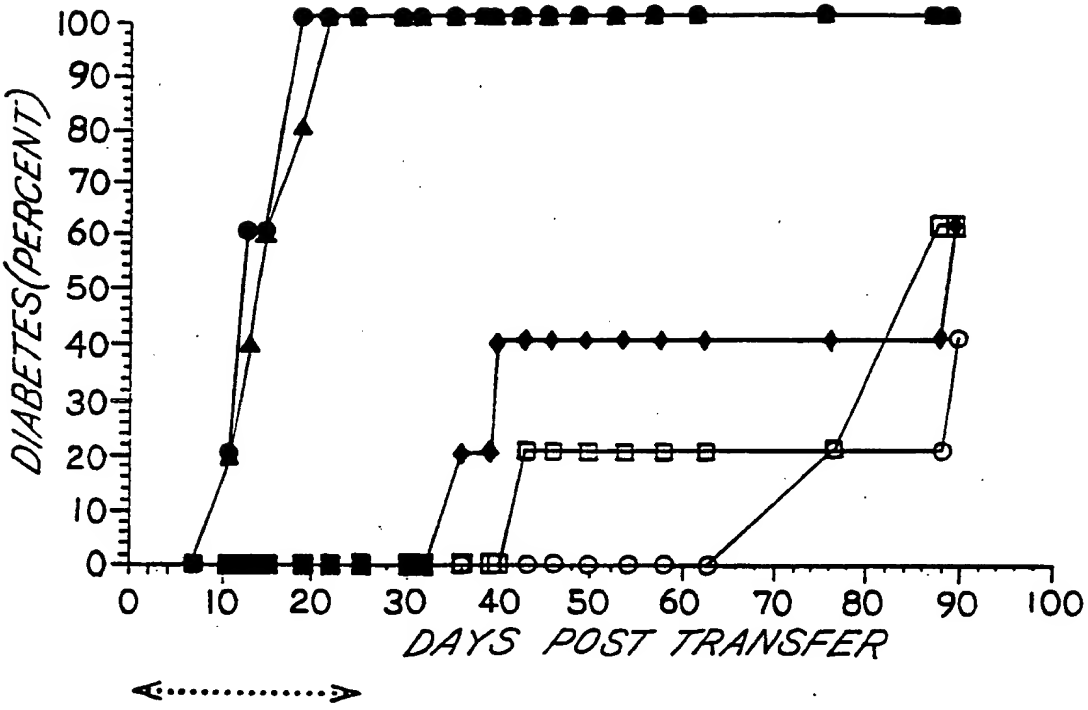

DIABETES(PERCENT)
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
DAYS POST TRANSFER

FIG.4

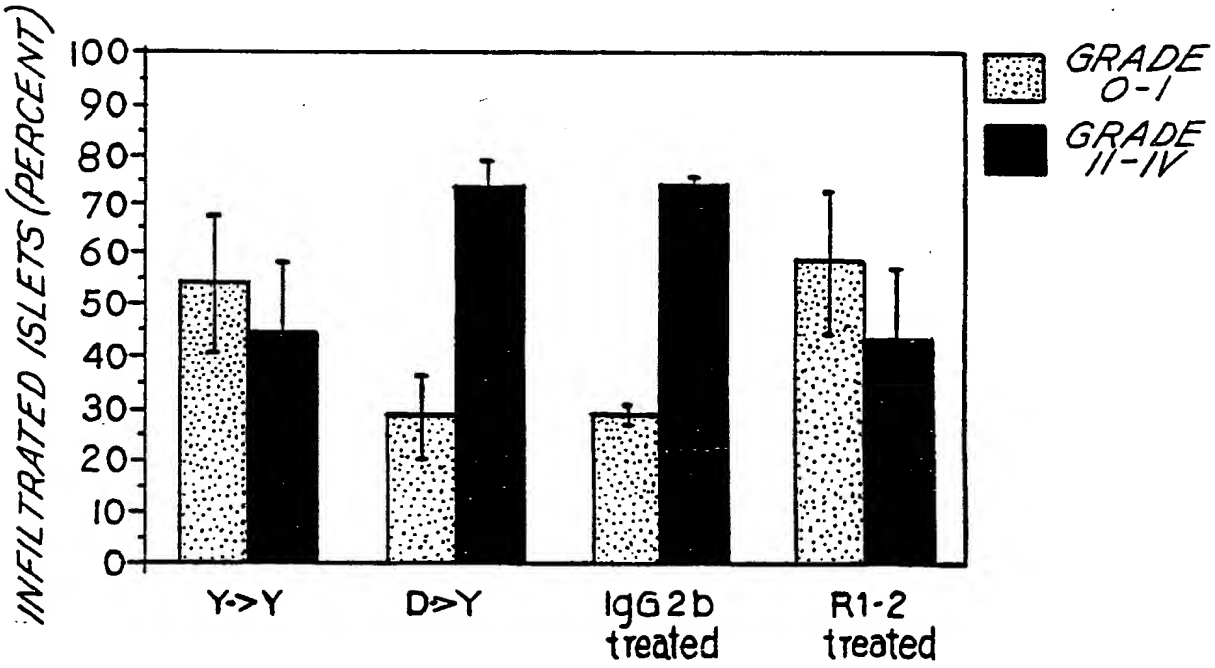
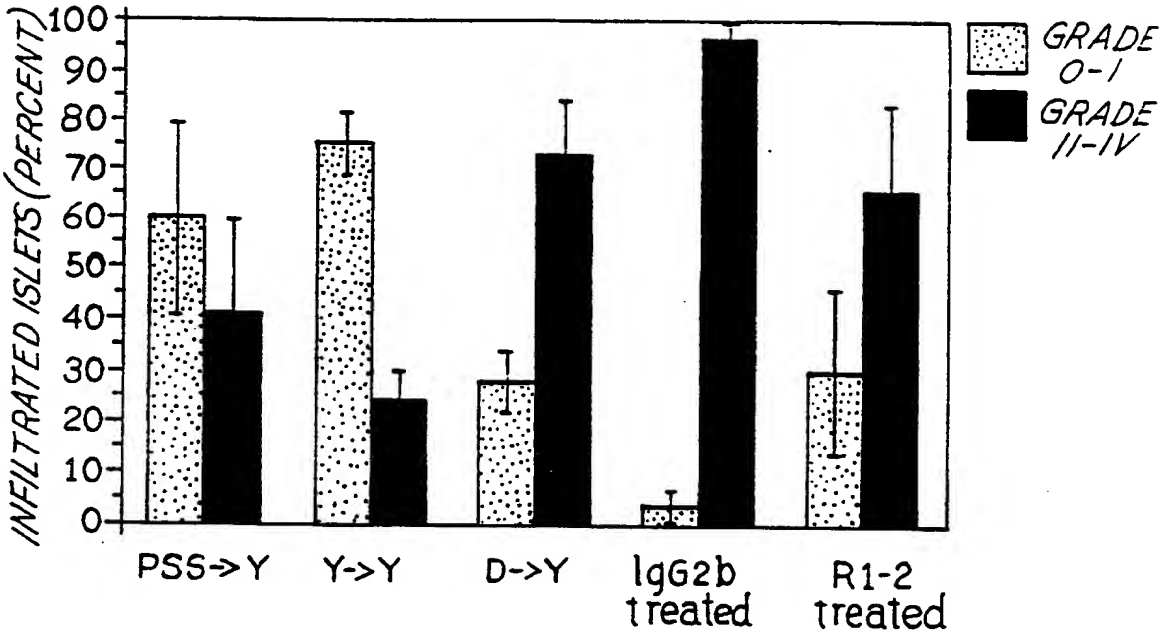

INFILTRATED ISLETS (PERCENT)
100
90
80
70
60
50
40
30
20
10
0
Y->Y
D>Y
IgG2b treated
R1-2 treated
GRADE 0-I
GRADE II-IV

FIG. 5


INFILTRATED ISLETS (PERCENT)
100
90
80
70
60
50
40
30
20
10
0
PSS->Y
Y->Y
D->Y
IgG2b treated
R1-2 treated
GRADE 0-I
GRADE II-IV

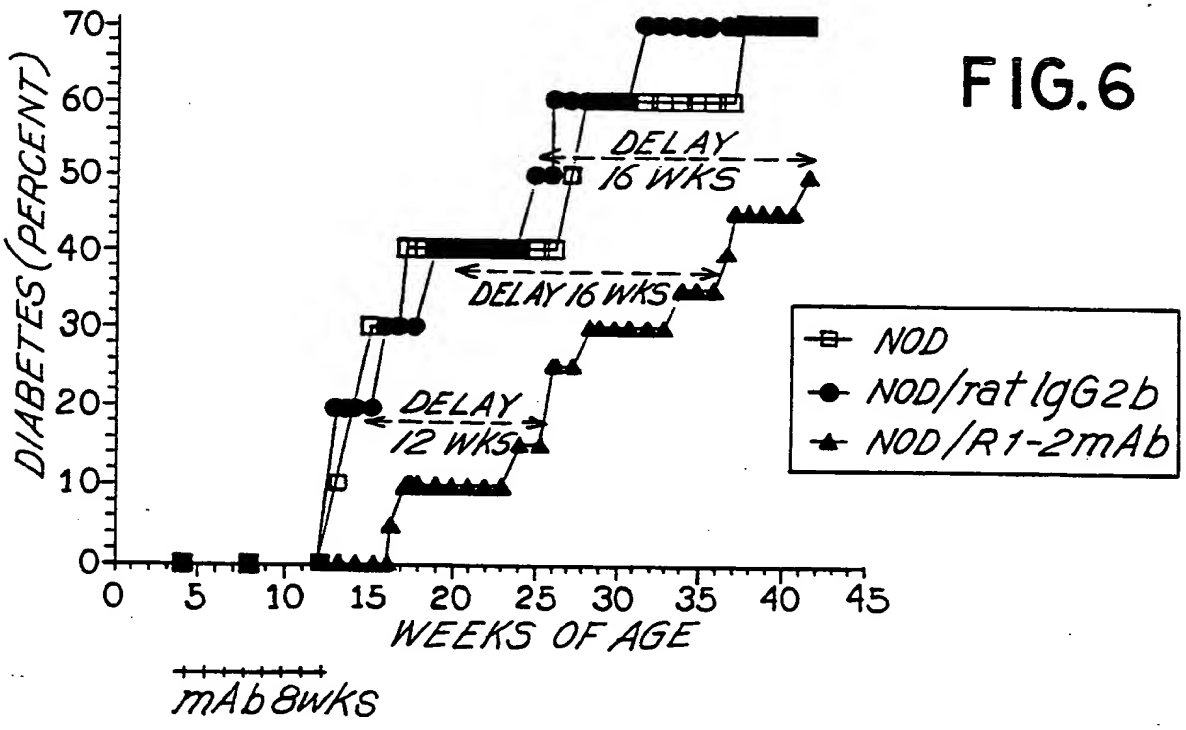

DIABETES (PERCENT)
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
WEEKS OF AGE
DELAY 16 WKS
DELAY 16 WKS
DELAY 12 WKS
mAb 8wks
NOD
NOD/rat IgG2b
NOD/R1-2mAb

FIG. 6

FIG. 7

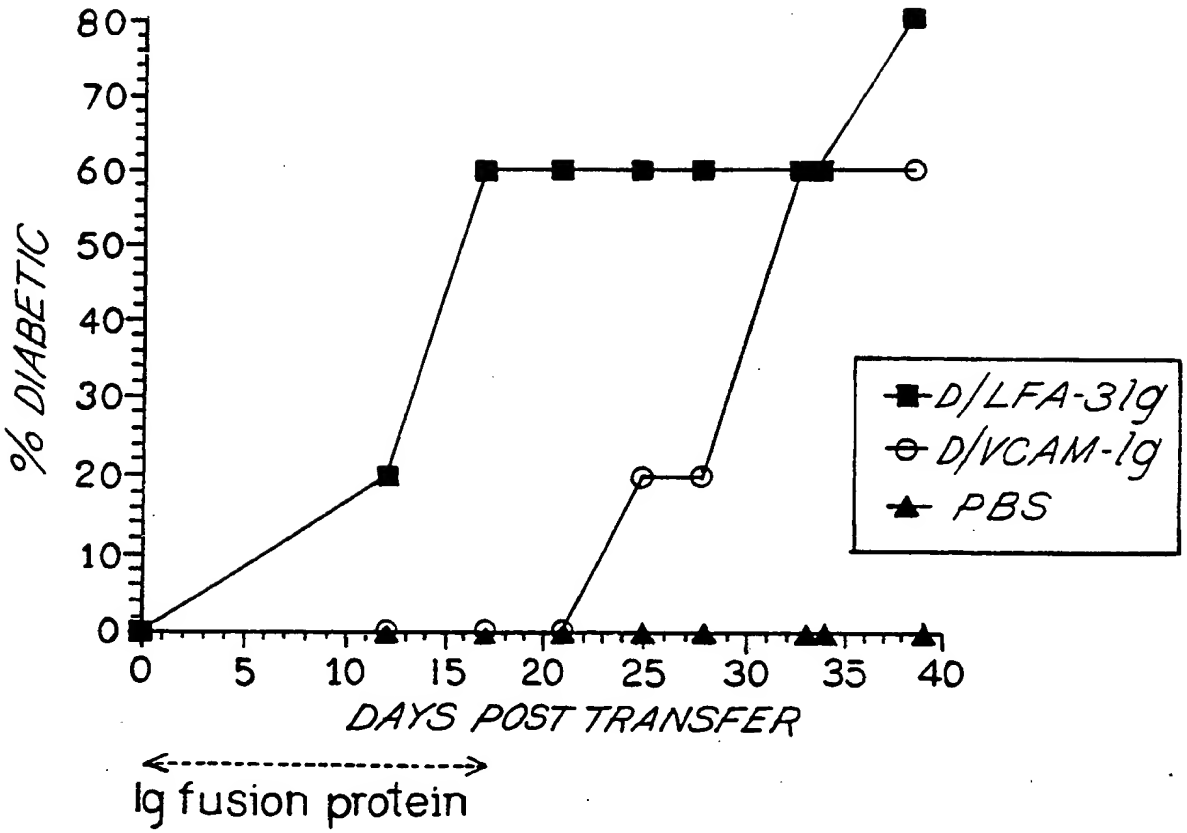
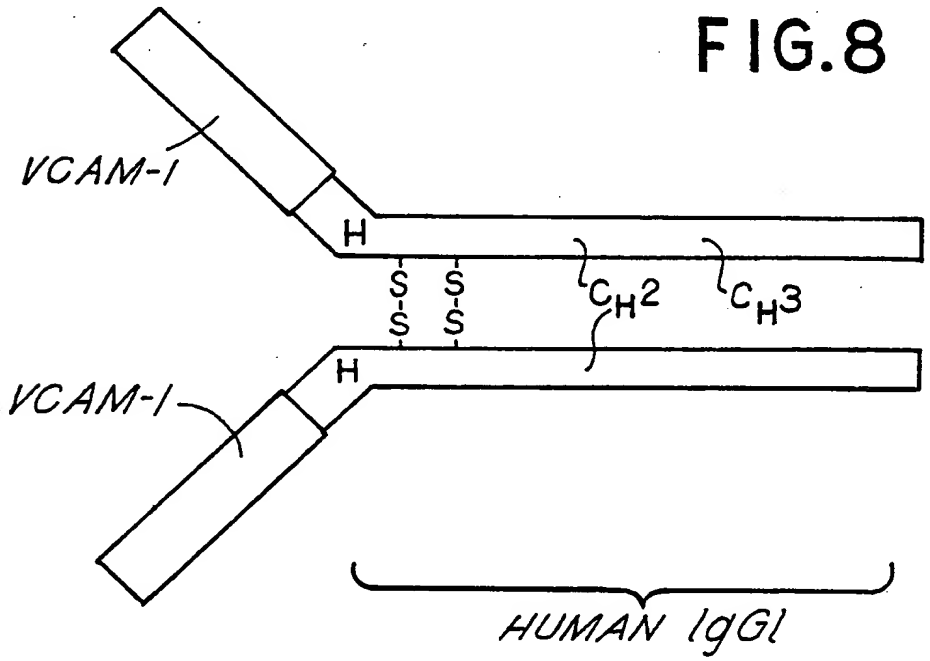

% DIABETIC
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40
DAYS POST TRANSFER
D/LFA-3Ig
D/VCAM-Ig
PBS
Ig fusion protein

FIG. 8


VCAM-I
H
S
S
S
S
$C_{H}2$
$C_{H}3$
VCAM-I
H
HUMAN IgG1